A B S T R A C T

Results from inoculated F_1, F_2, and F_3 generations of diallel crosses between five resistant and one susceptible variety of barley (Junior) indicated that four different genes for resistance to Ustilago hordei were involved. These were tentatively designated as follows: Uh, a dominant gene present in Titan and probably in O.A.C.21, Ogalitsu, and Anoidium; Uh 2, a dominant gene for resistance in Anoidium; uh 3, a recessive gene for resistance in Ogalitsu; and uh 4, a recessive gene responsible for the resistance of Jet. Interpretation of the results was complicated by skewed F_2 and F_3 distributions resulting from seedling mortality of severely infected plants. Chi-square tests for independence of morphological characters indicated that a gene for awn barbing is located in linkage group I. No association was found between inheritance of covered smut reaction and inheritance of number of kernel rows, lemma teeth, lemma and pericarp color, hull adherence, aleurone color, awn barbing, length of rachilla hairs, or rachis pubescence.

THE UNIVERSITY OF ALBERTA

INHERITANCE OF REACTION TO USTILAGO HORDEI (PERS.) LAGERH. IN CULTIVATED BARLEY

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A C K N O W L E D G M E N T S

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INTRODUCTION

The genetic basis of reaction to infection by Ustilago hordei (Pers.) Lagerh., incitant of covered smut of barley, has not been established. Several workers have studied the problem, but have been unable to obtain sufficiently high infection of susceptible material to classify their results in a satisfactory manner.

Early seeding and effective seed treatments have accomplished an important reduction in the incidence of covered smut in barley fields in western Canada in recent years. However the disease is still prevalent. It was present in ten to thirty per cent of the barley fields examined in Alberta during the years 1951 to 1953, in amounts ranging from traces to thirty per cent (5,6). Consequently, resistance to covered smut would be desirable in new and improved varieties.

Information on the inheritance of resistance to covered smut would be useful to the barley breeder. The present study was undertaken to attempt to discover what genes for resistance are present in certain varieties; to find out whether or not a number of genes can be combined in a single variety; and to investigate linkage relationships between

resistance and morphological characters. Close linkage between resistance and an easily discernible morphological character would be very useful in a breeding program.

REVIEW OF LITERATURE

Genetic Studies

In the past, failures to establish the genetic basis of covered smut resistance in barley have usually been due to poor infection, resulting in inability to differentiate resistant from susceptible segregants. Johnston (12) inoculated F_3 lines of a cross between Glabron, resistant, and Trebi, moderately susceptible to the collection he used. Segregation was not sufficiently clear to establish the number of genes involved in the inheritance of disease reaction. He found a low correlation between smut reaction and height of plant, but no correlation between smut reaction and awn barbing or smut reaction and earliness of heading.

Woodward and Tingey (32) inoculated two F_2 hybrid families with covered smut but the amount of infection was too low to permit a genetic interpretation. Pugsley and Vines (15) studied the reaction of F_3 progenies from the cross between Cape (susceptible) and Kwan (resistant) to a collection similar to Tapke's race 5. Their results could not be interpreted on a genic basis. They concluded that Kwan contained more than two dominant genes for resistance to covered smut.

Physiologic Specialization

The published information on physiologic specialization of the smut fungi has been reviewed by Christensen and Rodenhiser (4) and more recently by Holton (10). Therefore, only selected papers dealing with physiologic specialization in U. hordei will be reviewed here.

Faris (8) was the first to demonstrate physiologic specialization in U. hordei. He identified five pathogenic races in 1924. Rodenhiser (18) later identified seven distinct forms by cultural characteristics. Because of difficulty in obtaining infections, information on pathogenicity was obtained on only two of these. They differed pathogenically on the varieties Lion and Himalaya.

Aamodt and Johnston (1) found evidence of two physiologic races in 1935. Semeniuk (19) studied the pathogenicity of twelve collections on four varieties, obtaining evidence of four races. Further tests with the four collections gave conflicting results. In one test the differences between three of the collections practically disappeared.

In 1937 Tapke (22) reported the results from inoculations of the varieties Excelsior, Hannchen, Lion, Nepal, Pannier, Gatami, Odessa and Trebi with two hundred collections of U. hordei. All collections were made in the United States. He distinguished eight races by differences in pathogenicity on the first five varieties. Race 6 was most widely distributed and most frequently collected of the races found.

In 1945 Tapke (26) identified five additional physiologic races. The variety Himalaya was substituted for Gatami, otherwise his differential hosts remained unchanged. Races 1, 5 and 6 made up 86.5% of the total collections. Race 6 was usually predominant in areas

where varieties of the Manchuria - Oberbrucker types were most commonly grown. He found minor differences in size and color of chlamydospores, smoothness of spore walls, compactness of smutted heads and spore masses, degree of destruction of awns and degree of exsertion of smutted heads from the boot to be associated with different pathogenic races. However, none of these differences were sufficiently distinct for identification purposes.

Pugsley and Vines (15) tested three collections which resembled race 5 on Tapke's differential hosts. When tested on Australian varieties, minor differences were found between the collections. Cherewick (3) found all thirteen races in Canada. By adding White Hulless and Newal to Tapke's differential hosts he was able to identify 16 races.

Methods Of Inoculation

It has frequently been observed that the artificial dusting of barley seed with millions of spores is not an effective method of inoculation, while seed obtained from smutty barley fields usually yields a high proportion of diseased plants in the subsequent crop. Several investigators have attempted to develop effective inoculation techniques.

In 1923 Tisdale (31) found that hand-dehulled seed dusted with dry spores produced progenies with high infection percentages in a number of susceptible varieties. The hulled checks, both inoculated and uninoculated, gave poor results, the progeny of the inoculated seed being only slightly more affected than that of the uninoculated. Briggs (2) also found that seed dehulled by hand before inoculation with dry spores yielded a high percentage of infected plants, while hulled seed did not. However, he noted that stands from hand-dehulled

seed were reduced. In comparing progenies of seed lots dehulled by sulphuric acid with those of seed lots dehulled by hand, he found little or no difference in stand or percentage of infected plants. On the other hand, Johnston (12) found that dehulling with sulphuric acid resulted in high mortality and wide fluctuations in emergence of seed lots receiving identical treatments. It was his opinion that reduced stands from dehulled seed were the result of increased severity of infection by the pathogen. Aamodt and Johnston (1) found that dehulling by hand, with sulphuric acid or by scarification with sandpaper gave higher infections than inoculation of hulled seed. Inoculating hulled seed resulted in good stands with very little smut. Hand-dehulled seed produced somewhat lower stands with high proportions of infected plants. Acid-dehulled and scarified seed lots resulted in relatively poor stands and intermediate infection percentages.

In 1935 Tapke (21) suggested that effective inoculum was that which was under the hull. He developed a method of inoculation in which seed lots were shaken in a spore suspension, the suspension decanted, and the moist seed incubated for 16 to 20 hours at 20°C. This method resulted in high percentages of infected plants without appreciable reductions in stand. Tapke and Bever (29) later obtained slightly better results by subjecting the seed lots in the spore suspension to 30 inches of vacuum for 15 minutes. They obtained even better results by treating the seed before inoculation with a formaldehyde solution (1 to 320) for one hour, and washing and drying the seed before inoculation.

Tapke (25) made a comprehensive study of naturally inoculated seed lots and found that the most effective inoculum consisted of spores and mycelium from germinated spores on the pericarp of the caryopsis beneath the hull. He also observed that spores are not held intact in

the smutted heads in the field until threshing time. The membranes of the sori split a few days after the heads emerge, allowing some spores to escape and be carried to healthy heads. He concluded that dissemination of spores and inoculation of seed occurs through development, maturation, curing and drying of the standing or shocked grain, as well as in threshing. The progeny of samples taken before cutting contained 19.5% smutted heads; from the shock 27.1%; after threshing 50.6%; and after storage 71.3%. In storage, progressive increases in atmospheric humidities, up to the point where mold growth on the seed was appreciable, resulted in increases in the incidence of covered smut in the subsequent crop.

Woodward and Tingey (32) reported that seed dehulled by hand resulted in poorer emergence but higher infection than seed dehulled with sulphuric acid or not dehulled. Recently Popp and Cherewick (14) reported on a method which gave results equivalent to those obtained by hand-dehulling and dusting with dry spores. Seed to be treated was agitated violently in a spore suspension by means of a Waring Blendor. In their experiments this method was superior to Tapke's spore-suspension method.

Experiments by the author (unpublished) showed that plots grown from hand-dehulled seed inoculated by dusting with dry spores contained a higher proportion of infected plants than plots grown from hulled seed inoculated by Tapke's vacuum method. Stands from hand-dehulled seeds were lower than stands from hulled seeds, probably as a result of mechanical injury during the dehulling process. Furthermore, stands from hand-dehulled, inoculated seeds were usually lower than stands from hand-dehulled, uninoculated seeds. Evidently infection by the pathogen caused some seedling mortality. This seedling mortality varied

from variety to variety and from test to test.

Effect of Environment on Infection

Tapke (27) has reviewed the literature dealing with the effect of environment on infection by U. hordei. However, investigations having a direct bearing on the present study are discussed in the following paragraphs.

Faris (7) obtained the highest proportion of smutted plants in Hannchen barley at temperatures of 10 to 20°C. At 25°C, intermediate results were obtained, and at 5°C and 30°C, relatively few plants were infected. Seed lots grown in acid soil were about twice as badly infected as were those grown in alkaline soil. Soil moisture during germination had no pronounced effect. Faris obtained higher infections when temperatures were varied somewhat during the germination period.

In another study Faris (8) showed that environmental conditions after emergence are also important, and that successful infection may not occur even though the fungus has penetrated the host. Seed lots germinated under conditions favourable for infection were severely smutted when grown in the greenhouse, and were free from smut when grown in the field during the winter. Tapke (23) found that this was true for seed inoculated with dry spores, but found no effect due to environmental conditions after emergence on seed inoculated by the spore-suspension method. He concluded that since spores and mycelia become established beneath the hull by this latter method, penetration of the host takes place sooner and the fungus becomes sufficiently well established in the host tissue to be unaffected by subsequent environmental conditions. In a later paper, however, Tapke (28) did find that environment after

seedling emergence had an important effect. He also found that the optimum temperature for infection of Hannchen barley was 70°F, while that for Pannier was 60°F.

The author (unpublished) also found a differential reaction to covered smut among barley varieties which was due to environment. Identical tests were seeded in the field and in the greenhouse in the late spring. Mean soil temperatures in the field at a depth of three inches were 51°F. for the first week after seeding and 54°F. for the second. In the greenhouse, mean soil temperatures were 66°F. for the first week after seeding and 62°F. for the second. The average percentages of infected plants in the variety Plush were 50.0% for the field test and 61.7% for the greenhouse test. Prospect and Trebi, on the other hand, averaged 28.4% and 32.2% respectively in the field test and 10.7% and 16.5% respectively in the greenhouse test. Apparently the higher greenhouse temperatures resulted in increased susceptibility of Plush and decreased susceptibility of the other two varieties.

In other experiments Tapke (24) reported increases in the proportion of diseased barley plants by tamping or deepening the soil layer over the seed or by using a heavy soil. The incidence of covered smut was more than double when the early growth of fully emerged seedlings was retarded through pruning the roots.

In 1931 Taylor and Zehner (30) reported on the effect of depth of seeding on the incidence of covered smut in susceptible varieties over a four year period. In Tennessee Winter, the amount of covered smut varied from 0.2% to 6.6% when seeded at a depth of one-half inch. When seeded three inches deep the amount of smut varied from 8.9% to 23.0% in the subsequent crop.

Woodward and Tingey (32) found that depth and date of seeding produced variable results but showed no consistent influence on infection. They did find that barley grown on a soil of moderately low fertility was more highly infected than that on a fertile soil.

Jones and Seif El-Nasr (13) noted that barley broadcast on moist ground and plowed in contained six times as much covered smut as when harrowed in and irrigated. They suggested that since penetration of the host must take place between the time the coleoptile is exposed and the time it is ruptured, deep seeding allows more time for penetration by the fungus. They found that the time between exposure and rupture of the coleoptile was two days at a depth of 4 cm. and 4 days at a depth of 12 cm.

MATERIALS AND METHODS

The investigations reported herein were carried out at the Cereal Breeding Laboratory, Experimental Station, Lethbridge, Alberta.

All known physiologic races of Ustilago hordei have been found in Canada. Tapke reported that race 6 is usually predominant where the Manchuria-Oberbrucker barley types prevail. Most Canadian barleys are of this type. For this reason, and because race 6 gave clear-cut distinctions between resistance and susceptibility among the proposed parent varieties, it was considered particularly suitable for this study.

In order to check the behaviour of race 6 under Lethbridge conditions, it was tested on Tapke's differential hosts. One test was sown in the field in 1953. Two replications were grown in rows ten feet long and one foot apart with 100 seeds in each row. A second test was

sown in the greenhouse in the spring of 1954 along with those crosses, described below, which involved Junior. Ten replications were sown in rows thirty inches long and three inches apart, with thirty seeds in each row. In both tests hulled varieties were hand-dehulled before inoculation with dry spores. The data from these tests are presented in the following section on "Experimental Results".

The parents of the hybrid populations studied were O.A.C.21 (C.A.N.*1086), Titan (C.A.N.1164), Ogalitsu (C.I.**7152), Anoidium (C.I.7269), Jet (C.I.967) and Junior (C.A.N.766). Differences in morphological characters between the covered smut resistant varieties and Junior, the susceptible variety, are shown in Table 1. Where no information is given in the table the variety concerned does not differ from Junior with regard to that particular character.

TABLE 1.

Contrasting characters of the variety Junior and other parental varieties

Characters	Junior	O.A.C.21	Titan	Ogalitsu	Anoidium	Jet
Row number	Six-rowed					Two-rowed
Lemma	Toothed		Untoothed		Untoothed	
Lemma and pericarp	White					Black
Hull adherence	Hulless	Hulled	Hulled	Hulled	Hulled	
Aleurone	White	Blue			Blue	
Awns	Rough		Smooth	Semi-smooth	Smooth	
Rachilla hairs	Long	Short		Heterogeneous	Short	Short
Rachis edges	Pubescent		Non-pubescent			
Covered smut (race 6)	Susceptible	Resistant	Resistant	Resistant	Resistant	Resistant

* Canadian Accession Number
** U. S. Dept. Agr. Accession Number

Diallel crosses were made between the six parents, resulting in fifteen crosses. Part of the crossed seed from each population was sown without inoculation. Since the crosses were made in the greenhouse under difficult conditions, some selfing occurred. The progenies of self-fertilized seeds were identified and discarded. The F_1 plants were harvested and threshed individually. A portion of the seed from each F_1 plant was sown, again without inoculation. The resulting F_2 plants were harvested and threshed individually to provide seed for the F_3 generation. Thus the F_2 of each population consisted of a number of families, and the F_3 of descendants of the same families. All hybrid material was threshed by hand.

In crosses involving Junior, F_2 plants were classified for those morphological characters by which the parents differed. Their identity was retained through the F_3 generation and classifications were checked by examining the F_3 progenies. This procedure was especially valuable in classifying for aleurone color. The classification of F_2 plants was not reliable for this character, and F_2 results were not accepted unless corroborated by the F_3.

Previous studies, as well as the experience of other workers, indicated that the most desirable method of inoculation would be to dust dry spores on hand-dehulled seed. The term "dehull" is not strictly correct since it is only necessary to peel back the lemma from the germ end of the seed in order to expose the embryo. Since in the literature the term "hand-dehull" has come to mean merely the exposure of the embryo in this manner, it will be used in this sense.

Where necessary, seed lots to be inoculated were dehulled as above and placed in small coin envelopes. If possible, thirty seeds were placed in each envelope. In a few cases, less than thirty seeds

were available. A 1:1 mixture of chlamydospores and French chalk was introduced into each envelope and the whole shaken vigorously to effect complete coverage of all seeds.

The F_1, F_2, and F_3 generations of each cross were grown under comparable conditions, together with the appropriate parents as checks. The crosses with Junior were sown to a depth of three inches in ground beds in the greenhouse in rows thirty inches long and three inches apart. The F_1 generations, F_3 lines, and parents were sown in individual rows, while the F_2 of each cross occupied several rows. The resistant parent involved in each cross occurred in every fifteenth row of that cross; Junior in every thirtieth row throughout all crosses. Minimum soil temperatures were maintained at approximately 60°F. Daytime temperatures were higher but were kept below 80°F. Little variation in temperature was observed between beds.

Because of lack of greenhouse space, crosses between resistant varieties were grown under irrigation in the field. Rows were eight feet long and a foot apart. Seeding was accomplished with a Kemp V-belt seeder. Parental checks were seeded about every twenty rows. As a check on the degree of infection occurring in susceptible material, rows of Junior were seeded at intervals in each range.

Seeding was delayed until June 25 when soil temperatures ranged between a minimum of 57°F. and a maximum of 67°F. A few days of cool weather followed. The minimum temperature recorded was 51°F. in the early morning of June 29, with a maximum of 55°F. that day. Soil temperatures were probably too low for maximum infection during the germination period.

After heading, the plants in all rows were pulled and counted. The total number of plants and the number infected were recorded for each row. The percentage of infected plants was calculated to the nearest whole number.

EXPERIMENTAL RESULTS

Reactions Of Differential Hosts

The results obtained from inoculations of eight differential hosts with the race of U. hordei used in this study are presented in Table 2. The high proportions of infected plants in susceptible varieties indicates that conditions were favourable for maximum expression of the disease. The reactions of the differential hosts to this race were similar to those obtained by Tapke to race 6 (26).

TABLE 2.

Percentage of infected plants in barley differential hosts inoculated with U. hordei race 6

C.I. No.	Variety	Field, 1953	Greenhouse, 1954
1248	Excelsior	0.0	0.0
1312	Himalaya	0.0	0.0
531	Hannchen	44.0	94.8
923	Lion	2.6	47.4
595	Nepal	0.0	0.0
934	Odessa	51.9	99.1
1330	Pannier	0.0	0.0
936	Trebi	16.3	90.0

Inheritance Of Reaction To Covered Smut In Crosses with Junior

The reactions of parents, F_1, and F_2 generations of crosses involving Junior are shown in Table 3. The distributions of parental rows, F_2 rows, and F_3 progenies by five per cent infection class intervals in the same crosses are shown in Table 4.

Interpretations were based largely on F_3 distributions. In some crosses the F_2 ratios supported the F_3, while in others they did not. Little significance was attached to F_2 ratios as compared to F_3 since the former were based on the reaction of individual plants, while the latter were based on reactions of progeny rows. In Chi-square tests for goodness of fit of observed to expected F_2 results, the expected ratios were corrected on the basis of reaction of the susceptible parent, Junior. For example, the observed F_2 ratio of the cross Junior x Titan was 241 healthy to 49 infected plants. Assuming a single dominant gene for resistance, the theoretical ratio would be 217.5 resistant to 72.5 susceptible plants. However, only 69.2% of the plants of Junior were infected. Therefore only about that percentage of the F_2 plants of susceptible genotype would be expected to be infected. When 69.2% of the susceptible class is transferred to the resistant class, the expected ratio becomes 239.8 healthy to 50.2 infected plants. This ratio is very similar to the actual ratio.

Johnston (12) has suggested that barley seedlings infected with U. hordei may fail to emerge. In the present study there was evidence that infection by the pathogen was responsible for some seedling mortality. The average stand of the check rows of all resistant parents was 73%, while that of the check rows of the susceptible parent Junior

was 58%. Moreover, the average stand of the rows of each of the resistant parents was higher than that of comparable rows of Junior. This occurred in spite of the fact that the seed of four of the resistant parents had been hand-dehulled, a procedure which in itself results in some mechanical injury, and therefore reduced stands. The seed used for the check rows of Junior, on the other hand, was threshed by hand to avoid mechanical injury, and of course required no dehulling. Although not conclusive, this evidence suggests that stands of Junior were materially reduced as a result of infection by the pathogen.

TABLE 3

Percentage of infected plants of parents, F_1, and F_2 of crosses inoculated with race 6 of *U. hordei* and grown in the greenhouse

Material	Total plants	Healthy, No.	Infected, No.	Infected, %
Junior x O.A.C.21 F_1	18	18	0	0.0
Junior x O.A.C.21 F_2	349	312	37	10.6
Junior	149	33	116	77.8
O.A.C.21	369	366	3	0.8
Junior x Titan F_1	23	23	0	0.0
Junior x Titan F_2	290	241	49	16.9
Junior	172	53	119	69.2
Titan	387	387	0	0.0
Junior x Ogalitsu F_1	22	21	1	4.5
Junior x Ogalitsu F_2	395	379	16	4.0
Junior	154	38	116	75.3
Ogalitsu	333	333	0	0.0
Junior x Anoidium F_2	337	322	15	4.4
Junior	188	51	137	72.9
Anoidium	360	359	1	0.3
Junior x Jet F_1	13	5	8	61.5
Junior x Jet F_2	249	158	91	36.5
Junior	173	33	140	80.9
Jet	258	256	2	0.8

TABLE 4

Distribution of parental rows, F_2 rows, and F_3 progenies, in five per cent infection class intervals, of crosses inoculated with race 6 of U. hordei and grown in the greenhouse

Material	0	1 5	6 10	11 15	16 20	21 25	26 30	31 35	36 40	41 45	46 50	51 55	56 60	61 65	66 70	71 75	76 80	81 85	86 90	91 95	96 100	Total rows
Junior x O.A.C.21 F_2	2	4	4	1	2	1	0	0	1													15
Junior x O.A.C.21 F_3	46	16	19	16	19	3	7	5	1	7	5	2	0	1	6	3	2	3	1	0	1	163
Junior											1	0	0	0	1	0	2	3	0	1	1	9
O.A.C.21	13	1	1																			15
Junior x Titan F_2	1	2	1	2	2	3	1	2														14
Junior x Titan F_3	60	7	19	24	17	10	5	3	5	0	7	3	6	2	6	4	3	2	1	2	0	186
Junior									1	0	1	0	1	0	1	3	2	0	1			10
Titan	15																					15
Junior x Ogalitsu F_2	7	6	4	1																		18
Junior x Ogalitsu F_3	83	17	20	30	7	4	3	6	4	7	1	5	1	1	0	0	0	1				190
Junior													1	0	2	2	1	2	0	0	1	9
Ogalitsu	16																					16
Junior x Anoidium F_2	3	8	2	0	1																	14
Junior x Anoidium F_3	82	17	23	14	9	3	7	1	1	4	2	3	1									167
Junior										1	0	0	1	1	1	0	0	2	0	1	1	8
Anoidium	13	1																				14
Junior x Jet F_2					1	3	2	3	1	2	0	1	3									16
Junior x Jet F_3	35	15	21	13	14	9	7	8	7	4	5	2	2	5	0	1	2	3	0	0	1	154
Junior															1	3	2	1	3	1	1	12
Jet	13	0	1																			14
Junior (all crosses)									1	1	2	0	3	1	6	8	7	8	4	3	4	48

If seedling mortality of Junior was caused by smut infection, the susceptible F_3 lines should have been affected. In that event, analysis of the F_3 data would be expected to show an inverse relationship between stand and infection. Accordingly, per cent stand was calculated for each F_3 line. To eliminate zero values, 0.1 was added to each infection percentage. Values for per cent stand and per cent infection were converted to $\sin^2\theta$, and a correlation coefficient calculated for each cross. The following results were obtained:

Cross	r.
Junior x O.A.C.21	-0.186*
Junior x Titan	-0.258**
Junior x Ogalitsu	-0.095
Junior x Anoidium	-0.256**
Junior x Jet	-0.265**

It is apparent that susceptible lines tended to contain fewer plants than resistant lines, indicating that some susceptible plants were so severely infected that they failed to emerge. The negative association between stand and infection in the cross Junior x Ogalitsu, although not statistically significant, indicates that there was probably some loss of susceptible seedlings. Since seedling mortality might have disturbed the ratios, it was considered in the genetic interpretations of the results.

Junior x O.A.C.21

The resistant reaction of the F_1 of this cross and the low proportion of infected plants in the F_2 (Table 3) indicate that the resistance of O.A.C.21 is dominant. The distribution of the F_3 progeny rows (Table 4) suggests that a single dominant gene for resistance

* Significant at the 5% level.
** Significant at the 1% level.

conditioned smut reaction.

In classifying F_3 progeny rows for smut reaction, rows free from smut were considered resistant; those containing 25% or less infected plants as segregating; and those containing over 25% as susceptible. Theoretically, these are the most logical points of separation between the three classes when parental reactions, escapes from infection, and seedling mortality are considered. But since two of the 15 segregating F_2 rows contained no smutted plants, a few segregating F_3 lines might have been included in the resistant category. Furthermore, since two of the 15 rows of the resistant check O.A.C.21 contained some smut, some resistant lines might have been classified as segregating. However, the number of lines misclassified in each case should have been approximately equal. Thus the ratio of resistant to segregating lines should not have been disturbed appreciably.

On this basis 46 F_3 lines were classified as homozygous resistant, 73 as segregating, and 44 as susceptible. This ratio fits the expected 1:2:1 ratio, with a Chi-square value of 1.82 and a P value of 0.40.

The F_2 ratio of 312 healthy to 37 infected plants did not fit the hypothesis, even when the expected ratio was corrected for escapes on the basis of the reaction of Junior. Mortality of infected seedlings would be expected to reduce the proportion of smutted plants in the F_2, and probably accounts for the lack of agreement between observed and expected ratios.

Junior x Titan

Table 3 shows that the F_1 of this cross was resistant, indicating that the resistance of Titan is dominant. The percentage of infected plants in the F_2 approached that which would be expected if a single dominant gene governed resistance. The distributions of the F_2 rows and F_3 lines shown in Table 4 support this assumption.

In classifying the F_3 lines for smut reaction, those free from smut were considered resistant, since the resistant parent Titan was smut-free. Lines in which up to 35% of the plants were infected were classed as segregating, corresponding to the distribution of the segregating F_2 rows. Lines containing more than 35% infected plants were classed as susceptible, conforming with the distribution of the susceptible parent Junior.

In this manner 60 lines were classified as resistant, 85 as segregating, and 41 as susceptible. A test of goodness of fit to a 1:2:1 ratio gave a Chi-square value of 5.26 with a P value between 0.10 and 0.05. This value for Chi-square is large, but within the limits of the 5% level of probability, indicating an acceptable fit. It is probable that some segregating lines fell into the resistant class because of seedling mortality, since there was a highly significant negative correlation between stand and infection among the F_3 progenies of this cross. This suggestion is supported by the fact that one of the 14 F_2 rows contained no infected plants. Hence it is probable that a similar portion of the segregating F_3 lines would be free from infection. When the expected F_3 ratio was corrected by transferring one-fourteenth of the segregating class to the resistant class, there was good agreement between observed and expected ratios. (Chi-square = 1.57 and P = 0.50).

The expected F_2 ratio, corrected for escapes, was 239.8 healthy to 50.2 infected plants. A Chi-square test for goodness of fit of the observed to the expected ratio gave a value of 0.035, corresponding to a P value of 0.90. This indicates close agreement between the observed and expected ratios. Thus, in this cross, the F_2 results corroborate the F_3.

Junior x Ogalitsu

The F_1 row of the cross Junior x Ogalitsu contained one infected plant. It is probable that this came from a self-fertilized floret of Junior. The low percentage of infected plants in the F_2, as well as the distribution of the F_2 and F_3 progenies, indicate that more than one factor for resistance was involved. A likely hypothesis is that two genes, one dominant and the other recessive, governed the inheritance of smut reaction.

In F_3, a dominant and a recessive gene for resistance would result in the following proportions of progenies:

7 homozygous resistant;
4 segregating in the ratio 13 resistant to 3 susceptible;
2 segregating in the ratio 3 resistant to 1 susceptible;
2 segregating in the ratio 1 resistant to 3 susceptible;
1 homozygous susceptible.

In classifying the F_3 progenies no attempt was made to separate lines segregating 13:3 from those segregating 3:1, since these classes could be expected to overlap. Similarly, lines segregating 1:3 were not separated from susceptible lines, again because of overlapping. Lines free from smut were considered resistant; lines containing from 1% to 25% infected plants were classed as segregating 13:3 or 3:1; and lines containing over 25% infected plants were classed as segregating 1:3 or susceptible.

The separation between resistant and segregating lines was made on the basis of the resistant parent Ogalitsu, which was free from

smut. The second separation was based on the premise that some lines segregating 3:1 would have contained as high as 25% infected plants. On the other hand, lines segregating 1:3 would be expected to show more than 25% infection. Susceptible lines should have fallen within the range of the distribution of Junior, which was above 25%.

When grouped according to these three categories, 83 lines fell in the first, 78 into the second, and 29 into the third. The test for goodness of fit of these results to the expected 7:6:3 ratio gave Chi-square value of 1.85, corresponding to a P value of 0.40. This indicates close agreement between the observed and the expected ratios.

The F_2 results did not support the hypothesis. The actual F_2 ratio of 379 healthy to 16 infected plants did not fit the expected 13:3 ratio, even when the latter was corrected for escapes on the basis of the reaction of Junior. Apparently seedling mortality affected the F_2 appreciably, since 7 of the 18 F_2 rows fell into the zero class. On the other hand, the classification of the F_3 lines should not have been influenced to any great extent, since the negative correlation between stand and infection was not significant. Therefore the theoretical F_3 classification was not corrected on the basis of the F_2 distribution.

Junior x Anoidium

The F_1 row of this cross contained several infected plants, as well as a plant of Junior that had escaped infection. When the cross was repeated and the F_1 tested for reaction to covered smut, it was found to be resistant. The F_2 (Table 3) and the F_3 (Table 4)

indicated that resistance was dominant and that more than one gene was involved.

It was assumed that two dominant genes for resistance governed covered smut reaction in this cross. Thus the following proportions of F_3 progenies would be expected:

7 homozygous resistant;
4 segregating in the ratio 15 resistant to 1 susceptible;
4 segregating in the ratio three resistant to 1 susceptible;
1 homozygous susceptible.

The F_3 progeny rows were classified as resistant, segregating, or susceptible. No attempt was made to separate the two groups of segregating lines since considerable overlapping would be expected. Smut-free lines were considered resistant; those containing from 1% to 30% infected plants were classed as segregating; and lines with more than 30% diseased plants were classed as susceptible. The separation between segregating and susceptible lines was based on the fact that there was a definite break in the distributions of two of the three individual F_3 families at the 30% point.

Classified in this manner, the observed ratio was 82 resistant to 73 segregating to 12 susceptible lines. When tested for goodness of fit to the theoretical 7:8:1 ratio a Chi-square value of 2.65 was obtained with a P value of 0.30, indicating a satisfactory fit.

Since three of the 14 segregating F_2 rows fell into the zero infection class, it is probable that a similar proportion of the lines segregating 15:1 were classed as resistant. On the other hand, because one of the fourteen rows of the resistant parent Anoidium contained some smut, a similar portion of the resistant lines

may have been classified as segregating. The theoretical ratio was corrected by transferring three-fourteenths of the lines segregating 15:1 to the resistant category, and one-fourteenth of the resistant class to the segregating category. When the observed ratio was tested for goodness of fit to the corrected ratio, a very close fit was obtained. (Chi-square = 0.49, P = 0.80).

The F_2 data from this cross support the hypothesis that resistance was governed by two dominant genes. The observed ratio was 322 healthy to 15 infected plants. The expected ratio, taking into consideration the reaction of Junior, was 321.6 to 15.4. The Chi-square test gave a value of 0.01 with a P value of 0.90, indicating an almost perfect fit.

Junior x Jet

The F_1 and F_2 data, shown in Table 3, indicate that resistance was recessive in this cross. The distribution of F_3 progenies shown in Table 4, on the other hand, resembles that which might be expected if resistance was governed by a single dominant gene. The most likely explanation of the results is that Jet carries a single recessive gene for resistance, and that the distribution of F_3 lines was skewed toward the resistant classes by seedling mortality of severely infected plants. The highly significant negative correlation between stand and infection ($r = -0.265$) supports this hypothesis. Susceptible plants which escaped infection would also tend to skew the distribution toward the resistant classes.

The F_3 lines were grouped into two classes; resistant, or segregating and susceptible. No separation was made between

segregating and susceptible lines. Thirty-five lines were classed as resistant and 119 as segregating or susceptible. When this ratio was tested for goodness of fit to the theoretical 1:3 ratio a Chi-square value of 0.43 was obtained with a P value of 0.50, indicating a good fit.

Since one of the 13 rows of the resistant parent Jet contained some smut, it is possible that some of the lines classified as resistant were actually segregating. When one-thirteenth of the resistant class was transferred to the segregating class, the expected ratio became (35.5):(118.5). This ratio is practically identical with the actual ratio.

The theoretical F_2 ratio, corrected for escapes on the basis of the reaction of Junior, was 98 smut-free to 151 smutted plants. This fits the actual F_2 ratio with a Chi-square value of 0.82 and a P value of 0.40. Thus the F_2 results support the interpretation of the F_3 data.

Tests Of Heterogeneity Of Crosses With Junior

In studies such as the present it is possible that the varieties used as parents are not genetically pure, and that the plants selected as parents from a particular variety are not of the same genotype. To determine whether or not this may have happened in this study, the separate crosses were tested for heterogeneity on the basis of individual F_3 families, using the method outlined by Goulden (9). The results of these tests are presented in Table 5. In all five crosses the total Chi-square and pooled Chi-square

values indicate that the data fit the assumed ratios. The heterogeneity Chi-square values indicate that the families are not heterogeneous. Therefore it is safe to assume that for any particular variety, the plants chosen as parents were of the same genotype.

TABLE 5

Chi-square values obtained by testing the heterogeneity of F_3 families

Cross	Assumed ratio	Total		Pooled		Heterogeneity	
		X^2	D.F.	X^2	D.F.	X^2	D.F.
Junior x O.A.C.21	3:1	7.04	5	0.35	1	6.69	4
Junior x Titan	3:1	5.62	7	0.80	1	4.82	6
Junior x Ogalitsu	13:3	2.45	6	1.51	1	0.94	5
Junior x Anoidium	15:1	4.70	3	0.26	1	4.44	2
Junior x Jet	1:3	4.05	5	0.43	1	3.62	4

One of the families of the cross Junior x O.A.C.21 did not fit the assumed 3:1 ratio. The Chi-square value obtained was 4.73, significant at the 5 per cent point. However the data from this family were included in the results since the deviation was probably due to chance. The ratios for all of the other families in all crosses did fit the assumed ratios.

Inheritance Of Reaction To Covered Smut In Crosses Between Resistant Parents

The reactions of parents, F_1, and F_2 in crosses between resistant varieties are presented in Table 6. The distributions of parental rows, F_2 rows, and F_3 progenies are presented in Table 7.

It will be noted that the F_1, F_2, and F_3 generations of the crosses among the four varieties O.A.C.21, Titan, Ogalitsu and

TABLE 6

Percentage of infected plants of parents, F_1, and F_2 of crosses inoculated with race 6 of *U. hordei* and grown in the field

Material	Total plants	No. infected	% infected
O.A.C.21 x Jet F_1	5	0	0
O.A.C.21 x Jet F_2	126	3	2.4
Titan x Jet F_1	21	0	0
Titan x Jet F_2	318	4	1.2
Ogalitsu x Jet F_1	3	0	0
Ogalitsu x Jet F_2	317	12	3.8
Anoidium x Jet F_1	18	0	0
Anoidium x Jet F_2	281	2	0.7
Titan x O.A.C.21 F_1	19	0	0
Titan x O.A.C.21 F_2	413	0	0
Ogalitsu x O.A.C.21 F_1	23	0	0
Ogalitsu x O.A.C.21 F_2	292	0	0
O.A.C.21 x Anoidium F_1	25	0	0
O.A.C.21 x Anoidium F_2	342	0	0
Titan x Ogalitsu F_1	6	0	0
Titan x Ogalitsu F_2	423	0	0
Titan x Anoidium F_1	14	0	0
Titan x Anoidium F_2	340	0	0
Ogalitsu x Anoidium F_1	21	0	0
Ogalitsu x Anoidium F_2	436	0	0
O.A.C.21	365	3	0.8
Titan	456	0	0.0
Ogalitsu	330	0	0.0
Anoidium	334	0	0.0
Jet	247	1	0.4
Junior	228	164	71.9

TABLE 7

Distribution of parental rows, F_2 rows, and F_3 progenies, in five per cent infection class intervals, of crosses inoculated with race 6 of *U. hordei* and grown in the field

Material	0	1 -5	6 -10	11 -15	16 -20	21 -25	26 -30	31 -35	36 -40	41 -100	Total rows
O.A.C.21 x Jet F_2	4	1	1	1							7
O.A.C.21 x Jet F_3	50	8	8	4	1	2	1	1	1		76
Titan x Jet F_2	13	2	0	1							16
Titan x Jet F_3	72	6	11	7	3	1					100
Ogalitsu x Jet F_2	6	4	1	2							13
Ogalitsu x Jet F_3	75	11	8	3	1	2	0	1	0	1	102
Anoidium x Jet F_2	16	0	0	1							17
Anoidium x Jet F_3	113	4	3								120
Titan x O.A.C.21 F_2	18										18
Titan x O.A.C.21 F_3	112	2									114
Ogalitsu x O.A.C.21 F_2	15										15
Ogalitsu x O.A.C.21 F_3	120										120
O.A.C.21 x Anoidium F_2	16										16
O.A.C.21 x Anoidium F_3	85										85
Titan x Ogalitsu F_2	19										19
Titan x Ogalitsu F_3	78										78
Titan x Anoidium F_2	15										15
Titan x Anoidium F_3	113										113
Ogalitsu x Anoidium F_2	18										18
Ogalitsu x Anoidium F_3	119										119
O.A.C.21	15	3									18
Titan	20										20
Ogalitsu	19										19
Anoidium	19										19
Jet	17		1								18
Junior										12	12

Anoidium were all resistant. Although two of the F_3 progenies of the cross Titan x O.A.C.21 fell in the 1-5% infection class, these must be regarded as resistant since three of the O.A.C.21 checks also appeared in this class. Apparently these varieties all have one gene for resistance in common, or have different genes which are very closely linked.

Segregation took place, however, among the progeny of crosses between the resistant variety Jet and the other four resistant varieties. Obviously, the Jet gene for resistance is different from any of the genes present in the others.

Unfortunately, the results from these crosses were of little value for checking the interpretations of the data obtained from crosses involving Junior. Low soil temperatures during the early stages of growth probably resulted in a rather high proportion of escapes from infection by plants with susceptible genotypes. This would account for the unexpectedly high proportion of F_2 rows and F_3 lines containing few or no smutted plants. Furthermore, in view of the differential response to temperature among varieties noted by Tapke (28) and the author, and since Junior did not occur in any of the crosses, the reaction of Junior could not be used to distinguish segregating from susceptible F_3 lines. Therefore, the lines could not be classified according to the genotypes of the F_2 plants.

Association of Characters

Junior differed from each of the resistant parents in one or more morphological characters already assigned to a linkage group. Most of the characters are governed by single genes, according to several investigators, and most are independently inherited. Linkage

studies in barley have been summarized by Robertson and co-workers (16, 17) and more recently by Smith (20). Therefore the individual references will not be given here.

In this study an attempt was made to verify previous reports as to the mode of inheritance of these characters, as well as to check their relationships with one another and with the genes conditioning reaction to covered smut. Appropriate Chi-square tests were made to test goodness of fit for the individual characters, and independence where two characters were considered together. The Chi-square values obtained are summarized in Table 8.

Eight different morphological characters were studied, each occurring in one or more of the crosses. The character non-six-rowed vs. six-rowed is a marker for linkage group I. Toothed vs. untoothed lemma also occurs in this group. The marker for group II is black vs. white lemma and pericarp. Hulled vs. naked seed is a marker for group III. This group also contains a gene governing awn barbing and a complementary factor for blue aleurone. Blue vs. non-blue aleurone marks group IV. In group V rough vs. smooth awns is known to be associated with long vs. short-haired rachilla and pubescent vs. non-pubescent rachis. Unfortunately the parents did not differ by any characters known to be in the remaining two linkage groups.

Table 8 shows that in most cases inheritance of these characters was governed by a single gene. However in two of the three crosses where awn barbing was involved, two genes were apparently acting. In the other cross the parents differed by a single gene pair. Rachis pubescence also appeared to be conditioned by two genes.

In the test of independence of row number and smut reaction in the cross Junior x Jet, the Chi-square value was significant at the 5% point. However, when the data were grouped according to genotype of the F_2 plants, the Chi-square test showed that the characters were independent. Similarly, the apparent association between lemma teeth and smut reaction in the cross Junior x Titan could not be verified when the data were put on a genic basis.

Hull adherence was associated with aleurone color in the two crosses in which both characters could be studied. Recombination percentages, calculated by the maximum likelihood method (11), were 15.78 ± 3.17 for Junior x O.A.C.21 and 14.06 ± 3.20 for Junior x Anoidium. Evidently the gene governing aleurone color in these two crosses is the one designated Bl 2, in group III. The significant Chi-square value obtained for the test of independence of hull adherence and length of rachilla hairs in the cross Junior x Ogalitsu is undoubtedly due to chance. No other evidence was obtained that would indicate such a relationship, and it has never been reported.

Rachilla hair length was associated with awn barbing in two crosses, as would be expected from previously published results. In the cross Junior x Ogalitsu a recombination percentage of 18.24 ± 4.49 was obtained. In the cross Junior x Anoidium no recombination value was calculated. Apparently two factors were involved in the inheritance of awn barbing in this cross. Since F_3 plants were not classified for awn barbing the genotype of the F_2 plants could not be deduced, and linkage intensity could not be calculated. Awn barbing was also associated with rachis pubescence in the cross Junior x Titan.

TABLE 8

Summary of Chi-square tests for mode of inheritance and independence of characters in crosses with Junior

Characters and crosses	N	Assumption	X^2	D.F.
Junior x O.A.C.21				
Hulled vs. naked (Nn)	163	3:1	0.73	1
Long vs. short-haired rachilla (Ss)	163	3:1	3.78	1
Blue vs. non-blue aleurone (Bl bl)	162	3:1	2.37	1
Hull adherence, rachilla hairs	163	Indep.	0.02	1
Hull adherence, aleurone color	162	Indep.	63.32**	1
Rachilla hairs, aleurone color	162	Indep.	0.72	1
Smut reaction, hull adherence	163	Indep.	14.40	10
Smut reaction, rachilla hairs	163	Indep.	5.85	10
Smut reaction, aleurone color	162	Indep.	13.34	10
Junior x Titan				
Rough vs. smooth awns (Rr)	186	13:3	0.03	1
Hulled vs. naked (Nn)	186	3:1	0.01	1
Toothed vs. untoothed lemma (Gg)	186	3:1	1.21	1
Pubescent vs. non-pubescent rachis (Hr hr)	186	15:1	0.52	1
Awns, hull adherence	186	Indep.	0.48	1
Awns, lemma teeth	186	Indep.	4.98*	1
Awns, rachis pubescence	186	Indep.	67.68**	1
Hull adherence, lemma teeth	186	Indep.	2.69	1
Hull adherence, rachis pubescence	186	Indep.	0.97	1
Lemma teeth, rachis pubescence	186	Indep.	16.07**	1
Smut reaction, awns	186	Indep.	5.75	9
Smut reaction, hull adherence	186	Indep.	6.72	9
Smut reaction, lemma teeth	186	Indep.	18.67*	9
Smut reaction, rachis pubescence	186	Indep.	9.05	9
Junior x Ogalitsu				
Rough vs. smooth awns (Rr)	190	3:1	0.01	1
Hulled vs. naked (Nn)	190	3:1	0.07	1
Long vs. short-haired rachilla (Ss)	94	3:1	0.13	1
Awns, hull adherence	190	Indep.	1.90	1
Awns, rachilla hairs	94	Indep.	32.04**	1
Hull adherence, rachilla hairs	94	Indep.	5.28*	1
Smut reaction, awns	190	Indep.	9.03	7
Smut reaction, hull adherence	190	Indep.	6.45	7
Smut reaction, rachilla hairs	94	Indep.	3.48	6

TABLE 8

Summary of Chi-square tests for mode of inheritance and independence of characters in crosses with Junior (cont'd)

Characters and crosses	N	Assumption	X^2	D.F.
Junior x Anoidium				
Rough vs. smooth awn (Rr)	167	13:3	5.94*	1
Hulled vs. naked (Nn)	167	3:1	2.44	1
Toothed vs. untoothed lemma (Gg)	161	3:1	1.51	1
Long vs. short-haired rachilla (Ss)	167	3:1	1.62	1
Blue vs. non-blue aleurone (Bl bl)	167	3:1	2.44	1
Awns, hull adherence	167	Indep.	0.21	1
Awns, lemma teeth	161	Indep.	13.77**	1
Awns, rachilla hairs	167	Indep.	13.77**	1
Awns, aleurone color	167	Indep.	0.02	1
Hull adherence, lemma teeth	161	Indep.	3.64	1
Hull adherence, rachilla hairs	167	Indep.	0.12	1
Hull adherence, aleurone color	167	Indep.	64.68**	1
Lemma teeth, rachilla hairs	161	Indep.	0.02	1
Lemma teeth, aleurone color	161	Indep.	4.56*	1
Rachilla hairs, aleurone color	167	Indep.	0.12	1
Smut reaction, awns	167	Indep.	0.13	6
Smut reaction, hull adherence	167	Indep.	4.47	6
Smut reaction, lemma teeth	161	Indep.	2.85	6
Smut reaction, rachilla hairs	167	Indep.	9.55	6
Smut reaction, aleurone color	167	Indep.	9.39	6
Junior x Jet				
Non-six-rowed vs. six-rowed (Vv)	154	3:1	0.42	1
Black vs. white lemma and pericarp (Bb)	154	3:1	0.42	1
Long vs. short-haired rachilla (Ss)	154	3:1	0.06	1
Row number, color	154	Indep.	3.05	1
Row number, rachilla hairs	154	Indep.	3.72	1
Color, rachilla hairs	154	Indep.	0.02	1
Smut reaction, row number	154	Indep.	18.83*	8
Smut reaction, color	154	Indep.	11.04	8
Smut reaction, rachilla hairs	154	Indep.	8.13	8

* Significant at the 5% point.
** Significant at the 1% point.

Again a linkage value could not be calculated, since neither character was simply inherited. It appears that one of the genes governing rachis pubescence in this cross is the gene Hr 2, known to be in group V. There were no segregates with rough awns and non-pubescent rachises. This would be expected since previous workers have found the gene Hr 2 to be completely linked with the gene R for rough awns in group V.

Awn barbing was associated with lemma teeth in the two crosses where Titan and Anoidium were the resistant parents. Again no linkage intensities could be calculated.

D I S C U S S I O N

An interesting feature of the data obtained from crosses between Junior and resistant varieties is that, in every cross, the distributions of the F_2 rows and F_3 lines were skewed toward the resistant classes. Part of this can be attributed to escapes from infection, and, in four of the crosses, to dominance. However these two factors cannot account for all of the skewness.

The negative correlations between stand and infection among the F_3 lines of each of the crosses indicate that distributions were also affected by mortality of infected seedlings. Variations in the values obtained for r suggest that the progeny of some crosses was influenced more than the progeny of others. This could be the result

of environmental conditions, or of hereditary differences among the parental varieties. Unfortunately, it was not possible to calculate the effect of seedling mortality on the individual lines. Future studies on the inheritance of reaction to covered smut of barley should be conducted in such a way that disturbances resulting from seedling mortality can be measured.

Loss of susceptible plants tended to obscure interpretation of the results. In the four crosses where resistance was dominant, susceptible classes would not be expected to be appreciably disturbed by seedling mortality. On the other hand, in the cross in which resistance was recessive, it is the resistant class which would not be expected to be disturbed by seedling mortality. Therefore, when the F_3 lines in each cross were grouped into two classes only, the effect of seedling mortality should have been virtually eliminated. The total and pooled Chi-square values in Table 5 show that the observed ratios all fit the expected. Thus it may be concluded that the resistance of each of the varieties O.A.C.21 and Titan is governed by a single dominant gene; that of Ogalitsu by a dominant and a recessive; that of Anoidium by two dominant; and that of Jet by a single recessive.

No segregation occurred among the progeny of the crosses between the resistant parents O.A.C.21, Titan, Ogalitsu, and Anoidium. The genes for resistance carried by the first two varieties and one of the genes for resistance of the others must be allelic, or very closely linked. In crosses between Jet and the other resistant parents segregation for smut reaction occurred. The gene governing the resistance of Jet, therefore, is obviously different from any of the others.

Data from crosses grown in the greenhouse were much more reliable than data from crosses grown in the field. The difference illustrates the importance of controlled temperatures in studies of the inheritance of reaction to covered smut of barley. Unfavourable environmental conditions no doubt account for the failure of other workers to obtain results suited to genic analysis. In future studies of this problem, environmental conditions should be controlled within the limitations imposed by practical considerations.

Robertson et al. (17) suggested the symbol Uh uh for the character resistance vs. susceptibility to U. hordei. Up to the present no genes for resistance have definitely been identified and given a specific designation. The resistance gene carried by Titan was undoubtedly acting in Johnston's studies (12) and should be given the designation, Uh. It is probable that O.A.C.21, Ogalitsu and Anoidium also carry this gene. The second dominant gene of Anoidium is tentatively designated as Uh 2, the recessive gene of Ogalitsu as uh 3, and the gene conditioning the resistance of Jet as uh 4.

The associations between characters reported here agree fairly well with previous findings. The gene governing aleurone color in the crosses involving O.A.C.21 and Anoidium is undoubtedly the gene Bl 2 located in group III, since an association was found with hull adherence in each cross. The gene R for rough awns, located in group V, is apparently involved in crosses between Junior and Ogalitsu, Titan, and Anoidium. Where studied, the expected association was found between awn barbing and rachilla hairs. However, Junior apparently differed from Titan and Anoidium by two genes for awn barbing. The

location of the other gene is in question, but is probably in group I, since it was associated with lemma teeth in both crosses. The apparent association between lemma teeth and aleurone color in the cross with Anoidium was probably due to chance since the Chi-square value was significant at the 5% point only. No genes for awn barbing have been assigned to group I. However, two are known which have not been assigned to any linkage group.

Evidently one of the genes governing rachis pubescence was the gene Hr 2 in group V. Another, Hr, is located in group I. Since rachis pubescence was also associated with lemma teeth in the cross with Titan it is most probable that this is the gene involved here. This supports the idea that the second gene for awn barbing discussed above is also in group I.

Unfortunately no evidence of linkage was found between the genes conditioning smut reaction and those for morphological characters. This negative result may be explained by the fact that no characters were studied in groups IV, VI or VII. Furthermore, only two groups were represented in each of two crosses and three groups in each of the other three.

It is encouraging that each of the varieties Ogalitsu, Anoidium, and Jet carries a different gene for resistance. One or more of these can be combined with the gene carried by Titan. At the present time information is not complete with regard to the interaction of these genes for resistance and physiologic races of U. hordei. Therefore it is not yet possible to determine the best combination of genes for maximum resistance to known races.

SUMMARY

1. The mode of inheritance of reaction to race 6 of Ustilago hordei was studied in diallel crosses between five resistant and one susceptible variety of barley.

2. The resistant parents were O.A.C.21, Titan, Ogalitsu, Anoidium, and Jet. The susceptible parent was Junior.

3. The F_1, F_2, and F_3 generations of each cross, along with parental checks, were grown under comparable conditions.

4. Progenies of crosses with Junior were grown in the greenhouse under controlled conditions, while those of the resistant parents were grown in the field.

5. Seed to be inoculated was hand-dehulled and dusted with chlamydospores of the pathogen mixed with French chalk.

6. The phenotype of each F_2 plant in crosses between Junior and resistant varieties was recorded with regard to morphological characters by which the parents differed. These observations were checked for accuracy in the F_3 generation.

7. Plants in all rows were pulled and counted and the percentage of infection calculated on a plant basis.

8. Tests of heterogeneity were applied to F_3 lines of crosses with Junior. These showed that the families were homogeneous and, therefore, that the data could be combined for each cross.

9. Distributions of F_2 populations and F_3 lines were skewed toward the resistant classes to a degree not explicable on the basis

of dominance and escapes. This was explained on the basis of seedling mortality of plants with susceptible genotypes, caused by the death of severely infected plants in the seedling stage.

10. Resistance was found to be due to a single dominant gene in Titan and in O.A.C.21, two dominant genes in Anoidium, a dominant and a recessive in Ogalitsu, and a single recessive in Jet.

11. The varieties Titan, O.A.C.21, Ogalitsu and Anoidium were found to have one dominant gene for resistance in common, or different genes closely linked.

12. The following tentative designations were given the genes for resistance identified:

Uh to the gene present in Titan;
Uh 2 to the second dominant gene of Anoidium;
uh 3 to the recessive gene present in Ogalitsu;
uh 4 to the single recessive gene of Jet.

13. Tests for independence of characters were conducted on the F_3 progenies of crosses between resistant varieties and the susceptible parent. No associations were found between smut reaction and morphological characters studied.

14. Associations found indicated that:

the genes v*, G, Hr and a gene for rough awns were present in group I;
the gene B alone represented group II;
the genes n and Bl 2 were linked in group III;
the genes R, s and Hr 2 were in group V.

15. The genes V, Hr, Hr 2 and B were involved in one cross; G, Bl 2 and the unidentified gene for rough awns in two; R and s in three; and n in four crosses.

* The gene symbols in 14 and 15 are referred to in Table 8.

B I B L I O G R A P H Y

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